liquid-ordered and liquid-disordered coexistence region in DSPC/DOPC/POPC/Chol mixtures. By controlling lipid composition, we see distinct types of modulated liquid-liquid phase morphologies, including linear, irregular, and angular features in GUVs. These studies show that both the size and morphology of membrane rafts can be controlled by adjusting the composition and the type of low-melting lipid in mixtures with high-melting lipid and cholesterol.

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Particulate Material Effects on Pulmonary Surfactant Models

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Introduction

Pulmonary Surfactants (PS) are present in the air-liquid interface of lung alveoli. Its main function is to enhance the alveoli dilatational properties by lowering the surface tension of the system, as well as to prevent the collapse during the respiration process[1]. Particulate Material (PM) are exogenous particles which are related to some respiratory problems[2]. In the present work is reported the effects of those particles in system models of PS.

Methodology

Dipalmitoylphosphatidylcholine (DPPC) is the major component of PS and Cholesterol (Chol) is the most abundant neutral lipid. DPPC monolayers are used as models and the effects of PM and Chol were evaluated by using Oscillating Drop System (ODS) and Atomic Force Microscopy (AFM) techniques.

Results

Chol brought the Dilatational Elastic Modulus (E) to higher values evidencing an increase in the rigidity of the monolayers. Such effect is explained by the fact that Chol molecules act as space fillers turning the monolayers into more rigid structures. PM showed two different effects. The first one being the decrease of E at low PM concentration. The second one is the increase in E values which is believed to be a result of the adsorption of the particles to the monolayers.

Conclusions

PM and Chol were observed to provoke changes in some physicochemical properties of DPPC monolayers. PM as exogenous structures may cause problems to the regular functions of the PS as already reported.

References

1) Goerke, J. *Biochim Biophys Acta* **1998**, 19, 79-89.
2) Arbex, M. A. et al. *Jornal brasileiro de pneumologia* **2004**, 158-175.

1474-Pos Board B244

Vesicles and Phase Dynamics: Cross-Linking Effects

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We study lipid phase behavior using giant unilamellar vesicles to model cell membrane dynamics. In our system, we investigate the effects of cross-linking in the head groups position via biotinylated lipids, avidin, and its analogues. Cross-linking is the linking of two molecules (biotinylated lipids) via a cross-linking agent (avidin). Vesicles allow us to isolate the lipid rearrangement due to cross-linking, a common activity on cell surfaces. By comparing specific binding strength of the coupling and self-adhesion, we study the role that cross-linking plays in membrane behavior. Confocal microscopy gives us the ability to visualize the membrane dynamics. Using phase specific dyes, we study the changes that occur with the addition of a cross-linker to the system. Förster Resonance Energy Transfer (FRET) enables us to detect clustering on the submicron scale, beyond the limits of conventional microscopy. Using FRET we detect lipid rearrangement associated with the transition from one-phase vesicles to two-phase vesicles using two different fluorescent dyes, a donor and acceptor. Both techniques allow us to quantify the phase behavior due presence of the cross-linking agent. From this simple cross-linking system, we model membrane responses to protein complex formation and oligomerization.

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Reconstitution of Acyl Specific Phospholipid Remodeling by Purified Tafazzin In Vitro

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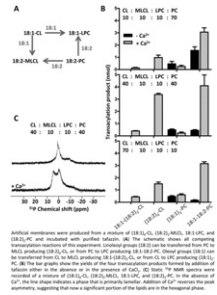
Cardiolipin is a mitochondrial phospholipid with a unique composition and distribution of acyl groups. The cardiolipin composition depends on tafazzin, a phospholipid-lysophospholipid transacylase, although the enzyme itself lacks acyl specificity. We incubated isolated tafazzin with various mixtures of phospholipids and lysophospholipids, characterized the lipid phase state by ^{31}P-NMR, and measured newly formed molecular species by mass spectrometry. Significant transacylation activity was observed only in non-bilayer lipid aggregates, in which lipids had a low packing order. The lipid phase state profoundly affected the substrate specificity of the tafazzin reaction. In particular, tetralinoleoyl-cardiolipin, a prototype molecular species, formed only under conditions that favor the inverted hexagonal phase. In isolated mitochondria, less than 2 percent of lipids participated in transacylations, suggesting that tafazzin acts only on privileged lipid domains. We propose that tafazzin reacts with non-bilayer lipids in mitochondria and that acyl specificity arises from spontaneous self-organization of these domains.



1476-Pos Board B246

Simulating Pores in Saturated Phosphatidylcholine Lipid Bilayers

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Lipid bilayers form the basic structure of cellular membranes. There is a large degree of diversity in the structure and composition of biological membranes. While one of the most important functions of membranes is to prohibit polar molecules from crossing the membrane, pore formation is crucial in a number of biological processes. We have used atomistic simulations to investigate the thermodynamics and kinetics of pore formation and dissipation in three saturated phosphatidylcholine bilayers, DLPC, DMPC, and DPPC. Pore formation has a large free energy cost, which increases as the tails length increases: 16 kJ/mol (DLPC), 40 kJ/mol (DMPC), and 80 kJ/mol (DPPC). We find that pore formation has a large unfavorable entropic contribution, possibly due to the constriction of water within the pore. The large unfavorable entropic contribution is compensated by a favorable enthalpic contribution to pore formation. Once formed, pores in the shorter lipid bilayers are larger and more stable than pores in bilayers with longer lipids. These results have broad implications on biological processes involving pore formation, such as lipid flip-flop, antimicrobial peptides, and cell penetrating peptides.

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Fluorinated Surfactants for Structural Studies of Membrane Proteins

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Membrane proteins are difficult to study in vitro. This is in particular related to their limited stability and motivates the search of new surfactants (e.g. 1-4), and among them, fluorinated and hemifluorinated (HFSs) surfactants. HFSs with a polymeric hydrophilic head proved to be particularly mild towards MPs (1). Surfactants were designed with chemically defined polar heads for structural applications. Lactobionamide derivative was found to be efficient in keeping several MPs water soluble and active. But it formed elongated rods (2). A new class of surfactants, the Glu- family, was synthesized, characterized in by neutron scattering (SANS) and analytical ultracentrifugation, and for its biochemical interest. The formation of rods is related to the low volumetric ratio between the polar head and hydrophobic tail. The surfactant bearing two Glucose moieties is the most promising one, leading to both homogeneous and stable complexes for both BR and the b6f. It was also shown be of particular interest for the structural investigation of membrane proteins using SANS (3).

(1) Breyton et al. (2004) FEBS Lett 564, 312-318.
(2) Lebaupain et al. (2006) Langmuir 22, 8881-8890.
(3) Breyton et al.(2009) Biophys. Journal. 97, 1077-86.
(4) Gohon et al. (2008) Biophys. Journal. 94, 3523-37.

1478-Pos Board B248

Nanoscale Imaging of the Piezoelectric Effect of Bilayer Phospholipid Molecules of Cell Membrane using Piezoresponse Force Microscopy*

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The dynamic piezoelectric effect of the plasma membrane and the nuclear envelope of rat A7r5 aorta smooth muscle cell is imaged with sub-3 nm spatial resolution using Piezoresponse Force Microscopy (PFM). The results verify that cell membrane is piezoelectrically active due to ordered arrangement of polar phospholipid molecules in the liquid crystalline state. A detailed analysis of the PFM signals with a 10 V / 0 V / −10 V DC bias voltage and a 10 V AC

voltage at a frequency of 2 kHz reveals piezoelectricity of cell membrane perpendicular to membrane surface Consequently, our results indicate that compression or tension of the membrane structure will lead to the change of membrane potential, suggesting the piezoelectricity of cell membrane plays an important role in physiological activities of cells such as cells communication and material transport.

This work is supported by the National Natural Science Foundation of China (No. 10804085 and 10774113).

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Secondary Structure Formation by Bilayer-Active Peptides Studied by Freeze-Fracture Electron Microscopy: From Disc Micelles to Cochleate Cylinder

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Melittin, a 26 amino acid, amphipatic peptide, is known for its hemolytic activity but some short-chain, cationic peptides show antimicrobial properties. Employing freeze-fracture transmission electron microscopy (ff-TEM) we investigated structural modifications within bilayers caused by these two membrane-active peptide groups. Ff-TEM as a cryofixation replica technique is a powerful tool to explore bilayer alterations in a probe-free mode down to a resolution limit of 2nm. Moreover, the fact that the fracture plane follows the area of weakest forces allows insides into the hydrophobic center of lipid bilayer [1]. Here we report the formation of disc micelles as a result of the reversible bilayer to micelle transformation caused by melittin in synthetic lipid bilayer [2]. Furthermore, we explored lipid domain formation in lipid films mimicking the lipid pattern of cytoplasmic membranes of Gram negative bacteria by arginine-rich antimicrobial peptides [3]. Additionally to lipid domains we observed secondary structure formation such as doughnut-type structures caused by arginine-rich peptides and cochleate cylinder (CCC) triggered by lysine-rich peptides. The new type of CCC has been proven to encapsulate and transport traditional, ineffective antibiotics such as erythromycin to resistant bacteria cells. The double-function of these antimicrobial peptides in forming CCC and promoting encapsulation and effective delivery of traditional antibiotics in/by these assemblies provides an interesting approach combating bacterial multidrug resistance [4].

References

[1] B. Papahadjopoulos-Sternberg, In: Liposomes: Methods & Protocols, Humana Press, 606 (2), 22 (2010) 333.
[2] C.E. Dempsey, B. Sternberg, BBA 1061 (1991) 175.
[3] R.M. Epand et al., BBA 1798 (2010) 1272.
[4] L. Levine et al. FASEB J. 24 (2010) 5092.

1480-Pos Board B250

Sequence-Specific Cargo Release from DNA Block Copolymers-Lipid Vesicles by Singlet Oxygen Formation

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The development of new bio-mimetic nanocontainers has multiple properties and applications in chemistry, biomedicine and nanotechnology. At this end, lipid vesicles have showed to be effective nanocontainers able to encapsulate a multitude of molecules successfully. However, the lipid membrane shows a poor chemical and physical specificity, what has been solved by functionalizing it with target-specific ligands as peptides, carbohydrates, antibodies, etc. Herein we present a new generation of liposomes based on the functionalization of their bilayer by anchoring single-strand DNA block copolymers (DBCs), such that free oligonucleotide (ODN) sequence can hybridize with its complementary strand. DBCs have been presented recently as a powerful tool in nanoscience because their high specifity.[1] The strong and highly specific hydrogen bonding of the DNA provides important functionality to liposomes and increase the number of potential applications in bioengineering and nanotechnology. In this work we present the successful results obtained in the stable incorporation of DBCs (consisted of 22-mer ODN linked to polypropylenoxide) in lipid membranes by a designed FRET experiment. Furthermore, this work shows some advances in the applications of functionalized DBC-lipid liposomes. We have increased the effectiveness of the usually disabled cargo release in lipid vesicles by the selective hybridization of DBC-lipid vesicles with a complementary DNA attached to a highly efficient photosensitizer. These photosensitizers are molecules that generate singlet oxygen ($^1O_2$) after light irradiation and which high reactivity provokes powerful damage on biological systems as lipid membranes. The designed hybridization architecture forces to the photosensitizer to be closer to the lipid bilayer, such that it is damaged by the $^1O_2$, giving rise to efficiently cargo release.

1. Alemdaroglu, F.E. et al. *Org Biomol Chem* **2007**, 5, 1311; Alemdaroglu, F.E. et al. *Angew Chem Int Edition* **2007**, 46, 1172.
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Effects Caused by Triton X-100 on Lipid Bilayers of Different Composition

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Detergents, such as Triton X-100, are widely used as solubilizing agents of biological membranes. Due to their amphiphilic character, detergents are incorporated in lipid bilayers at low concentrations, but are able to solubilize the membrane at higher concentrations. Here we study the incorporation of Triton X-100 into model membranes of different lipid composition and the subsequent membrane solubilization. The membranes were composed of pure POPC (palmitoyl oleoyl phoshatidylcholine), pure SM (sphingomyelin) and binary mixtures of these lipids with 30 mol% cholesterol. The process of incorporation/solubilization was followed at room temperature by optical microscopy of giant unilamellar vesicles (GUVs), isothermal titration calorimetry (ITC) and turbidity measurements. Optical microscopy revealed different responses of GUVs to Triton X-100, depending on membrane composition. Incorporation of Triton X-100 in the bilayer caused an increase in the surface area of GUVs composed of POPC, changes in the spontaneous curvature of GUVs of POPC/chol, and no morphological effects in GUVs made of SM and SM/chol. These observations were discussed in terms of a modulation of the flip-flop rate of bilayer-incorporated Triton X-100 by membrane phase and packing. Eventually, Triton X-100 was able to solubilize GUVs composed of POPC, POPC/chol and SM. During the solubilization process several holes opened and the bilayer gradually vanished. On the other hand, GUVs composed of SM/chol were insoluble in Triton X-100. ITC and turbidity measurements were used to determine the incorporation and solubility thresholds of Triton X-100 in the different membrane compositions. Financial support: INCT-FCx and FAPESP.

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Interface Specificity of Phospholipase Activity using a Novel Surface Dilution Kinetic Assay

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A novel assay using D-enantiomers of phospholipids as diluents for characterizing surface kinetics of lipid hydrolysis by phospholipases was employed to investigate interfacial phospholipase activity. The rationale of the method, verified in previous work, are: (i) D-enantiomers resist hydrolysis because of the stereoselectivity of the enzymes toward L-enantiomers and (ii) mixtures of L+D-lipids at various L:D ratios but constant L+D-lipid concentrations yield a surface dilution series of variable L-lipid concentration with constant medium properties. Kinetic characterization of bee-venom phospholipase A_2 activity at various types of interfaces including mixed (L+D)-lipid vesicles and bile salt + phospholipid aggregate-water interfaces was performed. The data were fit to a kinetic model and interface kinetic parameters were obtained. Activity was measured by fluorescence as well as pH-Stat methods. In the fluorescence method, the free fatty acid (FFA) binding protein, ADIFAB, was used. FFA is produced upon lipid hydrolytic breakdown. The fluorescence emission from the bound FFA-ADIFAB complex occurs at a longer wavelength than that from the unbound ADIFAB. The rate of hydrolysis is determined from the rate of increase of FFA bound ADIFAB fluorescence and decrease of unbound ADIFAB fluorescence. Activity data show excellent agreement with a kinetic model derived with D-enantiomers as diluents. Interface kinetic parameters show clear differences between different interfaces. The significant outcomes are (i) the novel assay itself; (ii) its ability to determine interface specific kinetic parameters and thereby (iii) characterization of interface specificity of lipolytic enzymes.

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Insights onto the Extraction of a Lipid from a Membrane using Molecular Dynamics Simulations

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In this study, we performed Molecular Dynamics simulations to determine the free energy profile in order to elucidate the interactions involved when extracting a lipid molecule from a lipid bilayer. Simulations of a POPC membrane containing a modified DOPE lipid (a model system for the biotinylated DOPE lipids used in AFM experiments) were performed using the Gromacs software package. The modified lipid molecule is first pulled out of the